Platelet Proteins, Including Platelet-derived Growth Factor, Specifically Depress a Subset of the Multiple Components of the Response Elicited by Glutathione in *Hydra*

Kazumitsu Hanai, Hisao Kato, Sachiko Matsuhashi,* Hiromichi Morita, Elaine W. Raines,‡ and Russell Ross‡

Department of Biology, Faculty of Science, Kyushu University, Fukuoka 812, Japan;
* Department of Biochemistry, Saga Medical School, Saga 840-01, Japan; and
‡ Department of Pathology, School of Medicine, University of Washington, Seattle, Washington 98195

Abstract. Human serum more strongly depressed the feeding response of *Hydra* (ball formation) elicited by S-methylglutathione than plasma. On the basis of the effect of several proteins released by platelets, at least five apparent components of the response (R1–R5) were suggested. Each of the platelet proteins examined specifically depressed a subset of these components. Among the platelet proteins examined, platelet-derived growth factor (PDGF) specifically depressed the R2 response (the concentration at which the depressing effect was 50% of the maximum [ED_{50}] was 0.17 pM), and basic fibroblast growth factor depressed the R3 and R5 responses (ED_{50} 0.50 aM) and the R2 response (ED_{50} 0.55 pM).

With respect to the depression of the R2 response by PDGF, addition of an anti-PDGF IgG or chemical reduction of PDGF, both of which prevent PDGF from binding to its cell surface receptor on responsive cells, eliminated the depressing effect of PDGF on the hydra response. The implications of these observations are discussed.

REDUCED glutathione elicits a feeding response in a small freshwater coelenterate, *Hydra* (29, 31). The response is quantitatively measured in duration of tentacle ball formation, a response associated with feeding (18). The response is depressed by lectins such as *Ulex europeus* I, *Ricinus communis* I and II (19), and by dopamine and related amines (20). These agents depress the response elicited by S-methylglutathione (GSM)[1] at concentrations <0.2 μM. The response elicited by GSM at higher concentrations (> 0.1 μM) are selectively eliminated, after the animals are illuminated by near UV light in the presence of S-(*p*-azidophenacyl)glutathione (21). These observations imply that there are multiple components of the response evoked at different concentrations of glutathione.

Although descendants of interstitial cells (nerve cells and nematocytes) may control the feeding response of *Hydra* (8, 28), the precise location of a receptor cell or glutathione receptor molecules mediating the response remains to be identified. Recently, while attempting to isolate a monoclonal antibody to the glutathione receptors, we found that serum caused a potent depression of the response. Since plasma depressed the response less potently, proteins released from platelets were evaluated to determine whether they were responsible for the depressing activity detected in serum. Here, we report that there are at least five apparent components of the response evoked by different concentrations of glutathione, and that individual platelet proteins, including platelet-derived growth factor (PDGF), specifically depress only certain subsets of these components.

The platelet proteins show many biological activities: an anti-heparin activity (platelet factor 4 [PF4], reference 33), chemotactic activities for fibroblasts (PF4 and β-thromboglobulin [βTG], reference 41). PDGF is a principal serum mitogen for connective tissue cells (2, 12, 23, 35–37), and also a chemoattractant for fibroblasts and smooth muscle cells (17, 42) as well as monocytes and nuetrophils (14, 46). Because of these activities, platelet proteins may play important roles in blood coaggulation and wound healing (39).

Materials and Methods

PDGF (human) was highly purified as previously described (35, 36). The molar concentrations of PDGF were calculated assuming 30,000 as its molecular weight. βTG and PF4 were purified from supernatant of frozen and thawed human platelets by heparin-Sepharose column chromatography (30). βTG was further purified by gel filtration on a column of TSK-Gel

Dr. Kato's present address is National Cardiovascular Center Research Institute, Suita, Osaka 565, Japan.

1. *Abbreviations used in this paper*: βTG, beta-thromboglobulin; ED_{50}, the concentration at which the depressing effect is 50% of the maximum; EGF, epidermal growth factor; FGF, fibroblast growth factor; GSM, S-methylglutathione; PDGF, platelet-derived growth factor; PF4, platelet factor 4.

G-3000SW (TSK America Inc., North Bend, WA). Their purity was examined by SDS PAGE and by amino acid analysis. They showed a single band on SDS PAGE in the presence or absence of 2-mercaptoethanol. Their amino acid compositions agreed with those expected from the published sequence of PF4 (13, 24) and βTG (4). A monospecific anti-PDGF IgG was prepared by Na_2SO_4 precipitation and DEAE-Sephacel chromatography of plasma from a goat immunized with purified PDGF. At a concentration of 20 μg/ml, the IgG completely antagonized the mitogenic activity of 1 ng/ml of purified PDGF on Swiss 3T3 cells. Highly purified preparation of basic fibroblast growth factor (FGF) from bovine pituitary (15) was a kind gift from Dr. A. Baird (The Salk Institute, San Diego, CA). Pooled human serum and plasma were a kind gift of Dr. T. Iwahashi (University Hospital of Kyushu University). Other reagents were all commercial products. Epidermal growth factor ([EGF] mouse submaxillary gland) was obtained from Biomedical Technologies (Cambridge, MA), goat anti-mouse-IgM IgG from Cappel Laboratories (Malvern, PA), and protamine sulfate from Nakarai Chemicals (Kyoto, Japan).

Assay of the Feeding Response of Hydra

10 animals of *Hydra japonica* were preincubated with a test substance (serum or purified platelet proteins) in 35-mm diameter dish containing 2 ml Pipes buffer (1 mM Pipes, 1 mM $CaCl_2$, pH 6.2). After 5 min of preincubation, a small amount of concentrated GSM was added into the medium to a specified final concentration, and the dish was gently swirled. GSM is as potent a stimulant as reduced glutathione, and more stable than the latter. The tentacles were motionless in the absence of GSM but, upon stimulation, they were shrunk and intertwined, resulting in a ball in the head region of the animal within 1 min (Fig. 1). The ball formation lasted for 10–20 min depending on stimulatory conditions. The response was determined as an average duration of the ball formation at 20°C as described in reference 18.

The depression of the response by a modulator was expressed as 100 − 100 × (response in the presence of a modulator)/(response in the absence of a modulator) %. The purified growth factor stock solution was diluted with Pipes buffer containing 2.5 mg/ml of bovine serum albumin (Sigma Chemical Co., St. Louis, MO) and a small amount of the diluted solution (<10 μl) was added into the medium. Bovine serum albumin had no effect on the feeding response of *Hydra* at concentrations <100 μg/ml.

Assay for Fibroblast Cell Growth

To examine the cell growth-stimulating activity of bovine sera, 10^5 cells of human fibroblast MRC-5 (American Type Culture Collection #CCL-171, reference 26, obtained from Flow Laboratories, Inc., MacLean, VA) were plated on 35-mm diameter plastic dish and cultured in Dulbecco's modified Eagle's medium (Difco Laboratories, Inc., Detroit, MI) supplemented with 5% bovine serum (fetal serum, lot 3E021, 99956; M. A. Bioproducts, Walkersville, MD; and neonatal serum, lot 60PB, PC27; Mitsubishi-Kasei Co., Tokyo, Japan) in humidified 5% CO_2. The medium was changed and the cell number was determined on days 3, 5, and 7.

Radioreceptor Assay of PDGF

The radioreceptor assay for PDGF was performed as described previously (6).

Results

A Depressing Effect of Serum on the Feeding Response of Hydra and Its Potency in Stimulating Cell Growth

Serum potently depressed the tentacle ball formation of *Hydra* elicited by GSM. The depressing activities of various lots of bovine serum are compared with their growth-promoting activity for human fibroblasts (MRC-5) in Table I. A correlation was noticeable between both the activities.

GSM elicited the feeding response at concentrations >10 nM, but at >100 μM, the response decreased. Pooled human serum (1%) greatly reduced the response in the whole range of GSM concentrations (Fig. 2). Though plasma also reduced the response to GSM of concentrations <1 μM and >20 μM, serum depressed the response in the whole stimulant concentrations at least 10 times more potently than plasma. For example, when the response was examined at 0.2 μM GSM, serum induced 50% depression at a concentration 20-fold lower than plasma (Fig. 3). Along with the correlation between the depression of the hydra response and the growth-promoting activity of various serum (Table I), the difference between plasma and serum (3, 27, 38) suggested the possibility that PDGF may be responsible for the de-

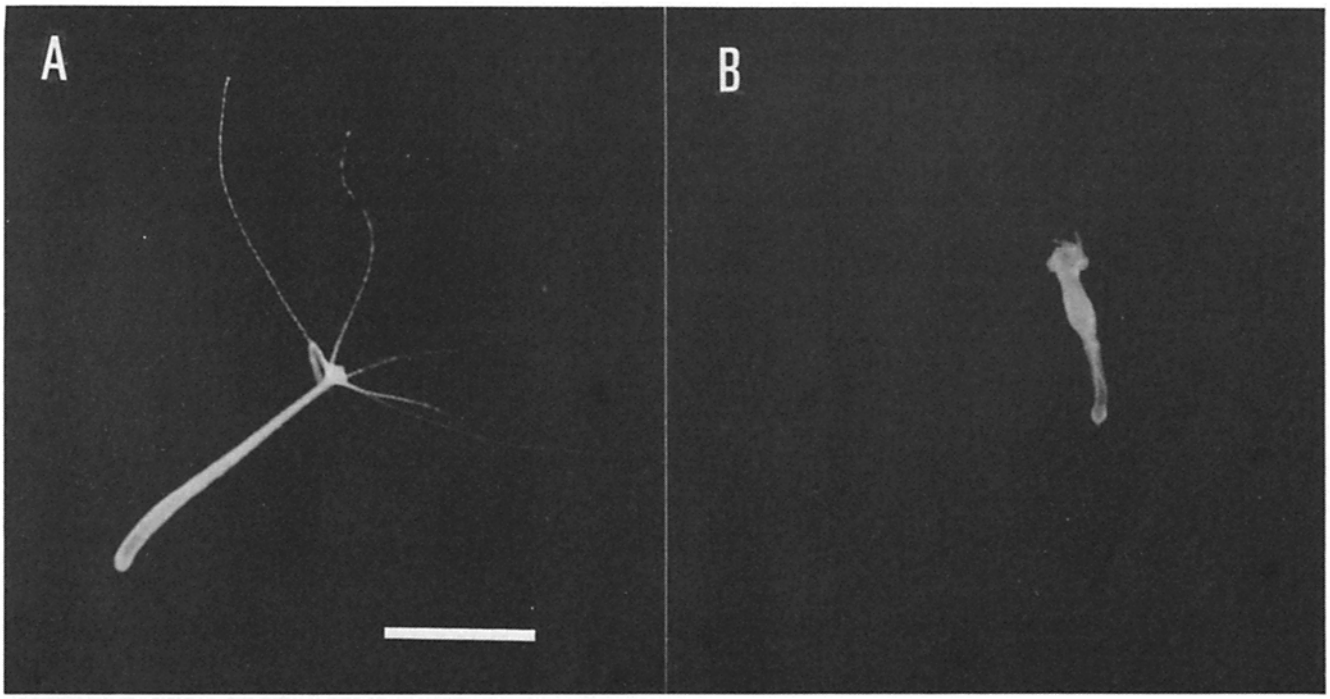

Figure 1. The feeding response of *Hydra japonica*. (*a*) The resting animal; the animal is relatively motionless and tentacles are stretched. (*b*) The animal stimulated with GSM; tentacles are folded and form a ball at the head region (tentacle ball formation). Bar, 2 mm.

Table I. The Depressing Activity of Various Lots of Bovine Sera on the Feeding Response of Hydra and their Growth-promoting Activities

Serum	Depressing activity*	Increase in cell number‡ After 3 d of culture	Increase in cell number‡ After 7 d of culture
		%	%
3E021 (Fetal)	17,100 ± 2,200	170 ± 13	920 ± 20
60PB (Neonatal)	8,800 ± 1,200	133 ± 25	585 ± 70
PC27 (Neonatal)	6,700 ± 800	120 ± 15	710 ± 60
99956 (Fetal)§	1,800 ± 500	106 ± 5	221 ± 23

* The depressing activity is expressed as the dilution of relevant serum at which the response of *Hydra* to 0.1 μM GSM is reduced to 50% of the response in the absence of serum. The activity was estimated from the depression at three to four different concentrations of relevant serum.
‡ The increase in cell number of MRC-5 fibroblasts after 3 and 7 d of culture supplemented with 5% of relevant serum is expressed as percent of the cells plated at day 0. The figures are the mean of two determinations.
§ A bottle of fetal serum 99956 that had deteriorated during usage in one of our laboratories was used. A new bottle of the same lot showed stronger depressing activity and supported better growth of MRC-5 cells.

pressing activity of human serum on the feeding response of *Hydra*.

The Depressing Effect of Alpha Granule Proteins

We examined the depressing effect of a supernatant of frozen and thawed human platelets fractionated on a heparin–Sepharose column, and found that a major active principle was eluted at the same position where PDGF activity was eluted (data not shown). We next examined purified PDGF, which is a major growth factor in serum released from alpha granules of platelets (reviewed in references 37, 39, and 45). Total depression of the response was not achieved with purified PDGF (Fig. 3). The concentration at which the depressing effect was 50% of the maximum (ED_{50}) was 0.17 pM for PDGF, which is ~100 times lower than the 33 pM required for half-maximal stimulation of [^{3}H]thymidine incorporation into Swiss 3T3 cells (for example, 36). This ED_{50} value is also far lower than the concentrations when PDGF acts as a chemoattractant (14, 17, 42, 46).

We examined the depressing effects of other proteins released from platelets on the response elicited by 0.1 μM GSM. EGF (34) and basic FGF (32) are also growth factors released from platelets and present in serum. They also depressed the response (Fig. 4). Total depression was not observed with either of these growth factors. The ED_{50} for basic FGF was 0.55 pM, assuming its molecular weight of 16,000 (15). The value is ~10-fold smaller than the amount required for half-maximal stimulation of cell growth (15). The ED_{50} for EGF was 15 nM, fivefold greater than typically required for stimulation of cell growth (9).

Two platelet proteins present in serum, PF4 and βTG, also depressed the response (Fig. 4). An ED_{50} of 15 nM was observed for βTG, 100-fold greater than needed for chemotactic activity for human fibroblasts (41). PF4 was only able to inhibit ~50% of the response with an ED_{50} of 20 nM, twofold greater than half-maximal chemotactic activity (41).

The Effect of Glutathione Concentration on the Depressing Activity of Platelet Proteins

Since the depressing activity of serum varies with the concentration of glutathione (Fig. 2), we examined the response

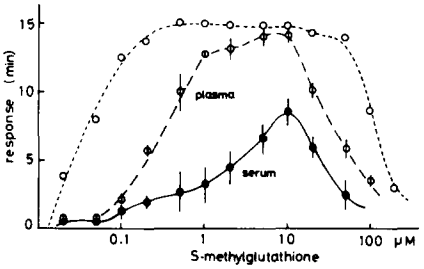

Figure 2. The effect of pooled human serum and plasma on the ball formation of *Hydra*. The response was determined at different concentrations of GSM in the absence of any additives (○), in the presence of 1% pooled human serum (●), and 1% pooled human plasma (ϕ). The response is an average duration (min) of the ball formation. Each point is an average of three determinations (30 animals). Bar is the standard deviation.

at different concentrations of stimulant in the presence of near saturating doses of each of the platelet proteins to examine their possible differential effects on the response (Fig. 5). In the presence of 3.3 pM of PDGF, only the response to GSM at concentrations from 0.1 to 1 μM was depressed, and the response below 0.05 μM or above 1 μM was not affected at all (Fig. 5 *A*). The same results were obtained in the presence of a 10-fold higher concentration of PDGF (data not shown). This observation suggests that PDGF specifically depresses a component of the response at concentrations from 0.1 to 1 μM.

Saturating amount of basic FGF (2.6 pM) depressed the response to GSM concentrations >0.1 μM (Fig. 5 *B*). Even the lower dose of basic FGF (0.26 fM) depressed the response to GSM above 1 μM to the same degree as the higher dose, whereas the response below 0.5 μM became near normal (Fig. 5 *B*). The ED_{50} of basic FGF for the response at 2 μM GSM is 0.50 aM (data not shown), 10^6-fold lower concentration than required at 0.1 μM GSM (Fig. 4).

A near saturating amount of EGF (83 nM) depressed the response to a degree similar to that of the higher dose of basic FGF (Fig. 5 *C*). A saturating amount of PF4 (170 nM) depressed the response to GSM concentrations <0.1 μM and >20 μM, and decreased the response to GSM concentrations between 0.2 and 10 μM by ~30% (Fig. 5 *D*). Near saturating amount of βTG (170 nM) greatly reduced the response to GSM at all concentrations examined (Fig. 5 *E*). The re-

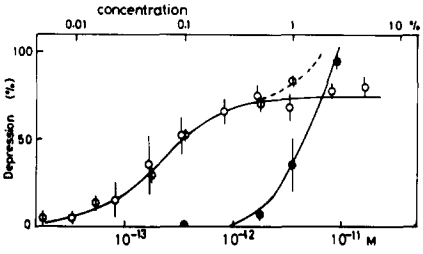

Figure 3. The depressing activity of pooled human serum, pooled human plasma, and the purified PDGF on the response of *Hydra*. The animals were stimulated with 0.2 μM GSM. Different concentrations (top abscissa) of pooled human serum (ϕ) or pooled human plasma (●), or different concentrations (bottom abscissa) of purified PDGF (○) were analyzed relative to their effect on the *Hydra* feeding response. Each point is an average of three to five determinations. Bar is the standard deviation.

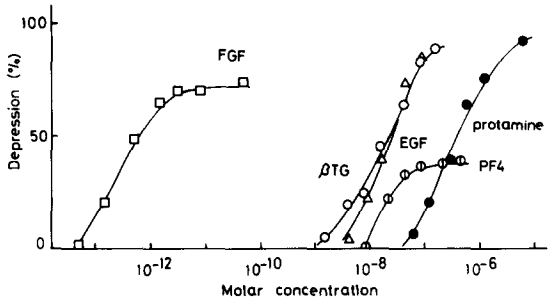

Figure 4. The depressing activity on the response of platelet alpha granule proteins and protamine. The response of *Hydra* was determined in the presence of different concentrations of basic FGF (□), EGF (△), purified human platelet βTG (○) and PF4 (ϕ), and an arginine-rich basic protein, protamine (●). The animals were stimulated with 0.1 μM GSM. Each point is an average of three to four determinations.

sponse observed at GSM concentrations ~10 μM was resistant to depression, as was observed for EGF and FGF.

The Effect of Modulators of PDGF Binding on the Depressing Activity

An anti-PDGF IgG, which inhibited PDGF binding to its cell surface receptor (Raines, E. W., and R. Ross, unpublished observations), eliminated the depression of PDGF but had no effect on the depressing activity of basic FGF, EGF, βTG, and PF4 (Table II). The anti-PDGF IgG alone at the same concentration did not affect the response to any extent. In contrast, a goat IgG prepared from anti-mouse-IgM serum did not eliminate the depression of PDGF at the same concentration, indicating a specific action of the anti-PDGF IgG on the depressing activity of PDGF.

Two other modulators for the interaction of PDGF with its receptor were also examined. Chemically reduced PDGF is unable to bind to its receptor (46) and is no longer mitogenic. Reduction of PDGF with mercaptoethanol abolished the ability of PDGF to depress the feeding response of *Hydra* (Table III). Mercaptoethanol alone did not affect the response. Protamine is an arginine-rich basic protein which is able to completely inhibit binding of ^{125}I-PDGF to its cell surface receptor at concentrations of 5 μM (25). This protein is also able to completely inhibit the response at a GSM concentration of 0.1 μM with an ED_{50} of 0.25 μM (Fig. 4).

The Depressing Activity of Animal Sera

In the above studies, PDGF was the most potent platelet protein acting on the response at 0.2 μM GSM (Figs. 3 and 4). It therefore appeared that the depressing activity at 0.2 μM GSM could be used to estimate the PDGF content in biological fluids. This is also suggested by the effect of anti-PDGF IgG on the depressing activity of animal sera. The anti-PDGF IgG eliminated the major part of the depressing effect of 0.1% human serum, and had a smaller effect on 1% serum (Table II). The PDGF content of various animal sera, both by the depression on the feeding response of *Hydra* and by the PDGF radioreceptor assay, are compared in Table IV.

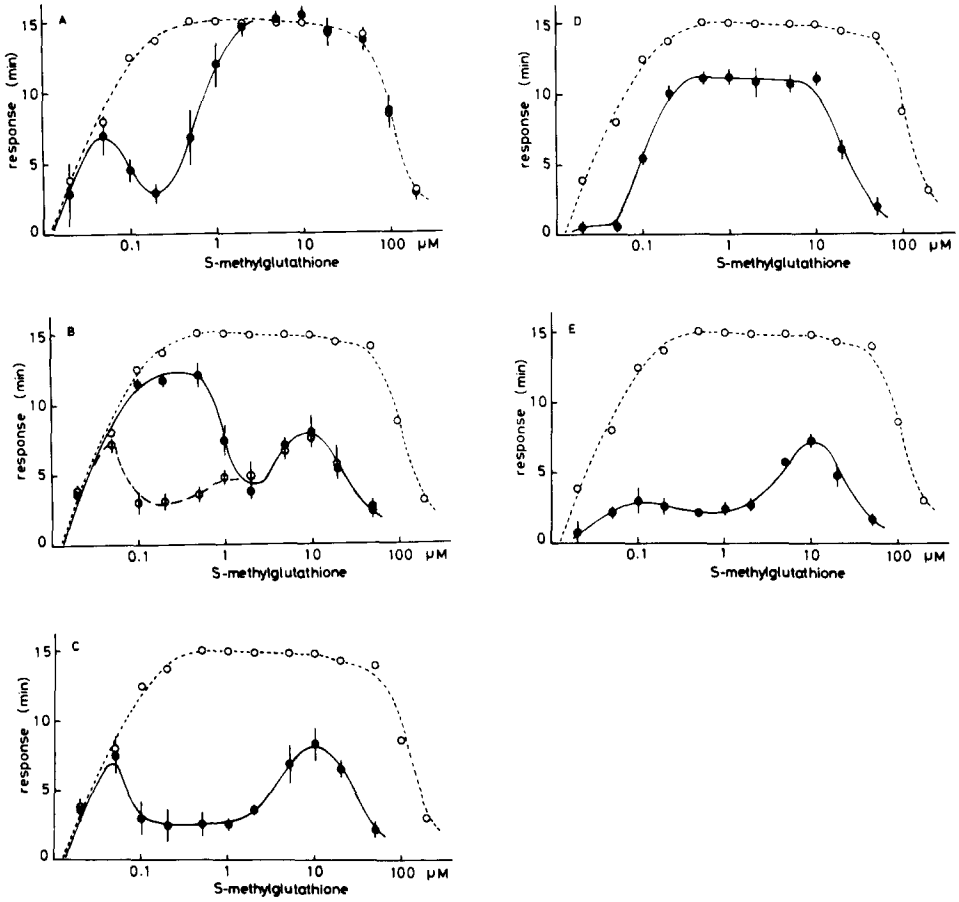

Figure 5. The effect of various platelet proteins on the response of *Hydra*. The response was determined at different concentrations of GSM in the absence of any additives (○) and in the presence of (*A*) 3.3 pM of purified PDGF (●); (*B*) basic FGF (ϕ, 2.6 pM; ●, 0.26 fM); (*C*) 83 nM of EGF (●); (*D*) 170 nM of PF4 (●); and (*E*) 170 nM of βTG (●). The response was determined as an average duration (min) of the ball formation. Each point is an average of three determinations. Bar is the standard deviation.

Table II. The Effect of Anti-PDGF IgG on the Depressing Activity of Alpha Granule Proteins and Biological Fluids

Additives		Depression*		
		Test	Plus anti-PDGF IgG‡	Plus control IgG‡
		%	%	%
None	–	0.0 (reference)	0.3 ± 6.3 (4)	ND
PDGF	33 pM	59.8 ± 6.5 (4)§	0.0 ± 1.5 (4)§	64.9 ± 1.7 (5)
Basic FGF	2.6 pM	69.6 ± 1.8 (6)	73.9 ± 4.9 (5)	ND
EGF	40 nM	77.6 ± 2.0 (3)	75.4 ± 11.8 (3)	ND
PF4	84 nM	45.1 ± 3.2 (3)	47.7 ± 0.7 (3)	ND
βTG	39 nM	50.3 ± 1.3 (3)	57.9 ± 6.7 (3)	ND
Human serum	0.1%	52.0 ± 3.3 (4)§	5.2 ± 4.3 (4)§	54.6 ± 6.4 (4)
	1%	83.4 ± 0.8 (4)§	32.3 ± 5.9 (4)§	ND

* The response was elicited by 0.1 μM GSM in the presence of alpha granule proteins and by 0.2 μM GSM in the presence of human serum. The figures are mean ± SD. The figure in parenthesis is the number of determinations.
‡ The anti-PDGF IgG was used at 41.9 μg/ml. The control IgG was an IgG fraction of goat anti-mouse-IgM serum and used at 42 μg/ml to demonstrate the specificity of anti-PDGF IgG. ND, not determined.
§ Significantly different ($P < 0.01$) by Student's t test.

The similar approximation by the two different assays, except for fetal bovine serum (see Discussion), suggests that the major depressing activity in animal sera detected in the hydra assay at 0.2 μM GSM may be due to PDGF.

Discussion

Multiple Components of the Response Elicited by Glutathione

From the present observations, at least five components of the response (R1–R5) (Fig. 6) are suggested. Each of them would be defined by a narrow range of stimulant concentrations: R1 at concentrations <0.1 μM, R2 from 0.1 to 1 μM, R3 from 0.5 to 5 μM, R4 from 2 to 20 μM, and R5 at concentrations >20 μM. The response at a specified concentration of GSM may be an integration of these components. The multiple components were also suggested by factors observed previously, such as a differential effect of dopamine on the response to GSM of different concentrations (20), and selective reduction of the response to GSM above 0.1 μM by in vivo photoaffinity labeling (21). Monoclonal antibodies that depressed only one of these components were also isolated (22).

In peripheral sensory systems, multiple receptor units, each of which is specialized in order to respond to a narrow range of stimuli covered by the whole sensory organ, have been observed. This is referred to as range fractionation (10). Glutathione response of *Hydra* might be a first example of range fractionation with respect to concentration of chemical stimulus. Further studies are required to clarify these multiple components of the response in cellular and molecular organizations, and their biological significance.

Table III. The Effect of Chemical Reduction of PDGF on the Depressing Activity of the Feeding Response of Hydra

Additives		Depression*
		%
Intact PDGF	0.33 pM	48.3 ± 6.5 (4)‡
Reduced PDGF§	0.33 pM	3.2 ± 3.4 (3)‡
Mercaptoethanol alone‖	–	1.3 ± 2.1 (3)

* The response was elicited by 0.1 μM GSM.
‡ Significantly different ($P < 0.01$) by Student's t test.
§ One part of purified PDGF solution (1 μg/ml) was mixed with nine parts of 10 mM mercaptoethanol, 50 mM Tris, pH 7.5. After a brief incubation at room temperature, the mixture was diluted to 10^5-fold with Pipes buffer for the behavioral assay. Because of a harmful effect of mercaptoethanol on the intact animals, a high dilution of mercaptoethanol, and thus a low concentration of PDGF, was used.
‖ The effect of mercaptoethanol alone at the same concentration used to reduce PDGF (see footnote §).

PDGF, FGF, and Other Platelet Proteins May Explain the Depressing Activity of Whole Serum

The depressing effects of platelet proteins are remarkable, especially considering the relatively weak effect of plasma proteins (Figs. 2 and 3). Bovine serum albumin, γ-globulins from cow, sheep, goat, and mouse, and bovine insulin showed very little or no effect at comparable concentrations (data not shown).

PDGF and basic FGF appear to be major components of the depressing activities of whole serum. Together with the effect of the anti-PDGF IgG on the depression of whole serum (Table II), the reasonable agreement of PDGF levels estimated by the depressing activities with those by the radio receptor assay (Table IV) indicates that PDGF is the major

Table IV. The PDGF Levels in Animal Sera Estimated by the Depressing Activity and by PDGF Radioreceptor Assay

Animal serum	PDGF estimation	
	By depression*	By radioreceptor assay‡
	ng/ml	*ng/ml*
Fetal bovine	204 ± 44	0.93 ± 0.20
Human	22.3 ± 9.4	20.4 ± 3.3
Bovine	1.2 ± 0.2	0.65 ± 0.11
Mouse	10.0 ± 3.8	11.9 ± 2.2
Horse	0.40 ± 0.1	0.17 ± 0.05

* The depression against the response elicited by 0.2 μM GSM was determined in the presence of three different doses of the relevant sample. The PDGF levels were determined from these depression values, as indicated in Fig. 3. Values are mean ± SD.
‡ The PDGF levels were determined by radioreceptor assay for the same samples as used for the depression assay.

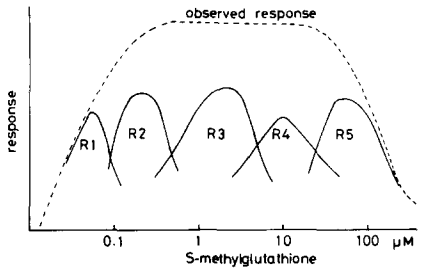

Figure 6. Five hypothetical components of the response elicited by glutathione. Each component (R1–R5) gives rise to a large response (——) only at concentrations of GSM in a narrow range. The observed response (- - - -) may be the integration of these components. Each line is drawn on the basis of the results of Fig. 5.

active principle in whole serum responsible for the depression of the R2 response. Basic FGF depressed the R3 and R5 10^6-fold more potently than it did the R2 response (Fig. 5 *B*). It appeared to be similarly potent for both R3 and R5 (data not shown). As the R3 response was depressed by basic FGF more specifically than the R5 response (Fig. 5 *B*), FGF activity may be estimated by the depression of the R3 response with less interference. EGF and βTG depress the R3 response only at high concentrations (Fig. 5, *C* and *F*, data not shown). We could estimate the basic FGF content to be 0.76 pg/ml by analyzing the depression of pooled human serum on the R3 response (data not shown).

The major component that depresses the R1 response is not clear at present. βTG and PF4 might be candidates, but an unknown component is likely, considering their levels in serum (6) and the relatively high concentrations required to depress the response.

Fetal bovine serum contained an activity potent enough to depress the R2 response (Tables I and IV). Though PDGF level in fetal bovine serum is low (0.83 ± 0.24 ng/ml, Raines, E. W., and R. Ross, unpublished observation and Table IV), the basic FGF-like activity, which was estimated from the depression on the R3 response, was high enough to explain its depressing activity on the R2 response (data not shown). The principal depressing activity of fetal bovine serum appears to be due to basic FGF or a related substance.

PDGF Modulation of the Feeding Response Appears to Be Receptor Mediated

The depressing activity of PDGF on the R2 response was modulated by all the investigated treatments that interfered with the interaction of PDGF with its cell surface receptor. A basic protein, protamine, potently depressed the feeding response at 10^5-fold higher concentrations than PDGF (Fig. 4, essentially the same depression was observed when the response was elicited by 0.2 μM GSM, data not shown). This is the same concentration difference as seen in competition between ^{125}I-PDGF and protamine for the cell surface receptor on responsive cells. The anti-PDGF IgG specifically eliminated the depressing effect of PDGF on the feeding response and this treatment prevented the binding of PDGF to its cell surface receptor (Raines, E. W., and R. Ross, unpublished observations). Finally, chemical reduction of PDGF, which destroys the ability of PDGF to bind to its cell surface receptor (46), also eliminated the depressing activity of PDGF on the feeding response. Together with the observation that PDGF modulates only a specific component of the response to glutathione, these results suggest that a specific PDGF receptor in *Hydra* modulates its feeding response. The protozoan *Tetrahymena* has also been reported to possess a PDGF receptor (1).

Platelet Protein Homologues in Lower Organisms and Biological Implications

Hydra has a strong regenerating potential when it is excised at its body column (44). Potent depressing activities were released from excised animals (Hanai, K., unpublished observations). Though further investigations are required, these activities may be due to platelet protein homologues in lower organisms. A phylogenetic analysis of clotted blood serum by radioreceptor assay found a PDGF homologue in the blood of all members of phylum Chordata, but nothing detectable below this phylum (43). However, the depression of the feeding response is more sensitive to the platelet protein homologues and appears to be less specific, even as to the R2 response, than the vertebrate PDGF receptor.

Immunoreactivities to neuropeptides such as FMRFamide, and oxytocin/vassopressin have been detected in hydra tissues (reviewed in 16). The head activator neuropeptide, which was first isolated from hydra tissues (40), was also found in human tissues (5). These observations suggest a primordial significance of peptides as information mediators in lower animals.

There would be a close relationship between feeding and growth. Feeding results in cellular proliferation, as indicated by a sharp, phasic increase of mitotic index in several cell types of *Hydra* (7, 11). That is, it seems likely that this process triggers the release of growth factors in addition to supplying nutrition. It is also possible that growth factors may directly participate in the regulation of food intake. The platelet protein homologues may play more multiple roles than their vertebrate counterparts.

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